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Phenotypic and Etiological Differences Between Psyllid Yellows and Zebra Chip Diseases of Potato

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Abstract Both potato psyllid yellows and zebra chip (ZC) potato diseases are associated with the potato psyllid, *Bactericera cockerelli* (Sulc). Aboveground plant symptoms of both diseases are similar but there is a difference in symptoms in potato tubers. ZC has recently been associated with a new species of the bacterium liberibacter, '*Candidatus* Liberibacter solanacearum', also known as '*Ca.* Liberibacter psyllaurous'. Mechanisms by which the potato psyllid might cause either ZC or potato psyllid yellows symptoms are not understood. Insect transmission studies were conducted to demonstrate psyllid vectoring of both diseases and to compare symptoms and development of the two diseases. Potato plants were exposed to both liberibacter-free and liberibacter-carrying potato psyllids and later evaluated for plant and tuber symptoms. These plants and tubers were then tested for liberibacter by polymerase chain reaction (PCR). In addition, potato plants exhibiting severe psyllid yellows/ ZC-like symptoms were collected from a commercial potato field heavily infested with the potato psyllid and tested for liberibacter. PCR detected '*Ca.* Liberibacter solanacearum' in ZC symptomatic plants and tubers resulting from exposure to liberibacter-carrying psyllids. Despite development of foliar symptoms that resemble those of ZC in plants exposed to liberibacter-free psyllids, no liberibacter was detected in these plants with psyllid yellows. Moreover, tubers from these plants with psyllid yellows did not exhibit any symptoms of ZC infection and tested negative for the bacterium. No liberibacter was detected in plants or tubers collected from the psyllid-infested potato field, suggesting that the observed symptoms were due to psyllid yellows. Furthermore, potato plants that were infected with liberibacter died sooner than plants that were infected with psyllid yellows. Although an association between liberibacter and ZC has been established, no pathogen is yet associated with potato psyllid yellows and mechanisms by which psyllid yellows symptoms are induced by the potato psyllid remain unclear.

Resumen Las dos enfermedades de la papa, el amarillamiento de la papa por psílidos y zebra chip (ZC), están asociadas con el psílido de la papa *Bactericera cockerelli* (Sulc). Los síntomas aéreos de la planta por ambas enfermedades son similares, pero hay una diferencia en los síntomas del tubérculo. La ZC se ha asociado recientemente con una nueva especie de bacteria liberibacter, "*Candidatus* Liberibacter solanacearum" , también conocida como "*Ca*. Liberibacter psyllaurous". No se han entendido los mecanismos por los cuales el psílido de la papa puede causar los síntomas, ya sea de la ZC o el amarillamiento de la papa por psílidos. Se condujeron estudios de la transmisión por insectos para demostrar la transmisión de ambas enfermedades por psílidos y para comparar los síntomas y el desarrollo de ambas enfermedades. Se expusieron plantas de papa a psílidos de la papa, tanto libres como con liberibacter, y se evaluaron posteriormente los síntomas de la planta y del tubérculo. Estas

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plantas y tubérculos se probaron después para liberibacter con la reacción en cadena de la polimerasa (PCR). Además, se colectaron plantas de papa que exhibieron síntomas severos de amarillamiento por psílidos/ZC, de un campo comercial de papa severamente infestado con el psílido de la papa, y se probaron para liberibacter. La PCR detectó "*Ca*. Liberibacter solanacearum" en plantas y tubérculos con síntomas de ZC, como resultado de su exposición a los psílidos con liberibacter. A pesar del desarrollo de síntomas foliares parecidos a los de ZC en plantas expuestas a los psílidos libres de liberibacter, no se detectó liberibacter en estas plantas con amarillamiento por psílidos. Aún mas, los tubérculos de estas plantas con amarillamiento por psílidos no exhibieron síntomas de infección por ZC y resultaron negativas a la bacteria. No se detectó liberibacter en plantas o tubérculos colectados del campo de papa infestado por psílidos, lo cual sugiere que los síntomas observados fueron debidos al amarillamiento por psílidos. Incluso, las plantas infectadas con liberibacter murieron más pronto que las infectadas con amarillamiento por psílidos. Aún cuando se ha establecido una asociación entre liberibacter y ZC, no se ha asociado a algún patógeno con el amarillamiento de la papa por psílidos y permanecen si aclararse los mecanismos por los cuales se inducen los síntomas del amarillamiento por el psílido de la papa.

Keywords Psyllid yellows · Zebra chip disease · Potato · Potato psyllid · *Candidatus* Liberibacter

Introduction

Potato psyllid yellows disease caused by feeding of the potato psyllid (*Bactericera cockerelli* Sulc) has been documented since the 1930s and is characterized by the yellowing or purpling of potato leaves and shoots, stunted shoots, aerial tubers and shortened and thickened internodes (Richards 1929, 1931; Eyer and Crawford 1933; Richards and Blood 1933; Eyer 1937; Wallis 1955; Arslan et al. 1985; Cranshaw 1994, 2001). Zebra chip (ZC), an emerging and damaging disease of potato produces aboveground plant symptoms that resemble those of potato psyllid yellows disease, but also severely affects potato tubers (Secor and Rivera-Varas 2004; Munyaneza et al. 2007a, b; 2008). Unlike potato psyllid yellows, ZC-infected tubers are characterized by a striped pattern of necrosis that becomes more pronounced when the potatoes are fried. Potato chips and fries processed from these infected tubers are commercially unacceptable (Munyaneza et al. 2007a, b; 2008).

ZC has been documented in the southwestern United States, Mexico, Central America, and most recently in New Zealand (Secor and Rivera-Varas 2004; Munyaneza et al. 2007a,b; 2008; Liefting et al. 2008) and has been associated with the potato psyllid (Munyaneza et al. 2007a,b; 2008). Also, recent studies conducted in the United States, New Zealand, and Mexico have shown the association of ZC with a new species of the bacterium liberibacter, '*Candidatus* Liberibacter solanacearum' also known as '*Ca.* Liberibacter psyllaurous', vectored by the potato psyllid (Liefting et al. 2008, 2009a,b; Abad et al. 2009; Crosslin and Bester 2009; Crosslin and Munyaneza 2009; Li et al. 2009; Lin et al. 2009; Munyaneza et al. 2009a,b,c; Secor et al. 2009). In addition, Hansen et al. (2008) recently reported identifying '*Ca.* Liberibacter psyllaurous' in potato plants exhibiting psyllid yellows symptoms. There have been reports of low or no ZC incidence in some potato fields heavily infested with the potato psyllid, raising the question of whether this insect pest is a real vector of ZC (Munyaneza, unpublished data). Recent studies have however indicated that some geographic populations of the potato psyllid may fail to induce ZC symptoms in potato tubers, but can still cause severe psyllid yellows-like symptoms in plants (Munyaneza et al. 2008). These non-ZC inducing psyllid colonies were later found to be free of liberibacter (Munyaneza, unpublished data). These observations suggest that the potato psyllid yellows disease may be different from ZC and could be caused by factors or/and agents other than the bacterium liberibacter.

It was long ago suggested that psyllid yellows disease is due to toxin injected into the plant by the potato psyllid while feeding (Carter 1939; Wallis 1955; Arslan et al. 1985; Cranshaw 1994, 2001). However, to date, the nature of the toxin has not been identified (Carter 1950; Daniels 1954; Abernathy 1991). It is essential that mechanisms by which the potato psyllid induces ZC and psyllid yellows disease symptoms in potato be understood. It is also important that similarities and differences between the two potato diseases be elucidated. The main objective of the present study was to conduct insect transmission studies to demonstrate potato psyllid vectoring of ZC and potato psyllid yellows diseases and to compare symptoms and development of the two diseases. Herein, we report the results of a series of experiments conducted under controlled conditions to compare symptom expression and etiology of the two diseases by exposing healthy potato plants to liberibacter-free and liberibacter-carrying potato psyllids. Furthermore, foliar tissue and tubers from field-collected potato plants exhibiting severe psyllid yellows-like symptoms were tested for liberibacter by polymerase chain reaction (PCR) to determine whether psyllid yellows disease was associated with this putative ZC causal pathogen.

Materials and Methods

Insect transmission experiments using potato psyllids were conducted in the summer and fall of 2008 under greenhouse conditions at USDA-ARS laboratories in Wapato and Prosser, WA. In addition, foliage and tubers from plants exhibiting psyllid yellows-like symptoms were collected in autumn 2008 from a commercial potato field in Dalhart, TX, brought to the laboratory to be examined for ZC symptoms and tested for the putative ZC causal pathogen '*Ca.* Liberibacter solanacearum'.

Sources of Potato Plants and Potato Psyllids

Certified pathogen-free potato mini-tubers (from tissue culture) of the variety Atlantic used in the study were obtained from CSS Farms Inc. (Colorado City, CO). This cultivar was selected for the transmission experiments because previous observations (Munyaneza et al. 2007a,b; 2008) indicated that this chipping variety is very susceptible to ZC and is commonly grown in the regions that are severely affected by the disease. Potatoes were planted at the USDA-ARS facility at Wapato, WA, in 1/2-liter pots (Kord Products, Toronto, Ontario, Canada) in a greenhouse maintained at 24–28ºC, 50±5% RH, and 16:8 (L:D) hr photoperiod. Growth media used for potatoes consisted of a mixture of 86% sand, 13.4% peat moss, 0.5% Apex time release fertilizer (J. R. Simplot Co., Lathrop, CA), and 0.1% Micromax micronutrients (Scotts Co., Marysville, OH). Growth medium's pH was adjusted to 6.8 through the addition of dolomite lime to optimize tuber germination and growth. Potato plants were in pre-bloom stage when used in the experiment and their height ranged from 15–25 cm.

Both liberibacter-free and liberibacter-carrying psyllid colonies were identified and collected from the field based on previous experiments (Munyaneza et al. 2008) and tested for '*Ca.* Liberibacter solanacearum' by PCR (Crosslin and Munyaneza 2009) to confirm the presence or absence of the bacterium. Insects were reared on potato plants in a controlled environmental room for several generations. The room was maintained at 29°C, 50% RH, and 16:8 (L:D) hr photoperiod. Prior to the transmission experiment, additional liberibacter-free psyllids were infected with the bacterium by allowing them to feed for at least a week on liberibacter-infected potato plants that had been established through grafting (Crosslin and Munyaneza 2009). To confirm that the insects were infected with '*Ca.* Liberibacter solanacearum', a subsample of psyllids was tested for the bacterium by PCR according to Crosslin and Munyaneza (2009).

Transmission Experiment

A transmission experiment was conducted in a greenhouse maintained at 24–28°C and 50±5% RH at the USDA-ARS facility in Wapato, WA. Liberibacter-free and liberibacter-carrying adults potato psyllids (20 insects/plant) were allowed to feed on individual healthy Atlantic potato plants for 30 days or continuously until the end of the experiment to test the hypothesis that potato plants fed upon by liberibacter-carrying potato psyllids would become infected with the bacterium and develop ZC symptoms, whereas those exposed to liberibacter-free psyllids will only develop psyllid yellows disease symptoms. One liberibacter-free potato psyllid colony and two different colonies of liberibacter-carrying potato psyllids were used in the study. One of the liberibacter-carrying psyllid colonies was originally collected from a ZC-affected field in southern Texas, whereas the second colony consisted of liberibacter-free psyllids that had fed directly on liberibacter-infected potato plant material produced through grafting (Crosslin and Munyaneza 2009). Plants without psyllids were used as controls. There were 15 plants per treatment. Where applicable, psyllid adults, nymphs, and eggs were removed from the plants after 30 days to lessen psyllid feeding damage and increase the chance for the plants to produce tubers, except in the treatment where liberibacter-free psyllids were allowed to stay and feed on the plants for the entire duration of the experiment (90 days). The psyllid adults were removed from the plants with an aspirator and later tested for liberibacter as described below. Nymphs and eggs were removed from the plants with insecticide sprays. After psyllid removal, all plants were transferred to a greenhouse maintained at 24–29°C, 60±5% RH, and 16:8 (L:D) hr photoperiod at the USDA-ARS facility in Prosser, WA, where environmental conditions conducive to ZC symptom expression had previously been observed (Crosslin and Munyaneza 2009). Plants were monitored for foliar ZC-psyllid yellows and tuber ZC symptom development and later tested for '*Ca.* Liberibacter solanacearum' by PCR to confirm ZC infection.

Source and Testing of Field-collected Potato Material

A potato field heavily infested with the potato psyllid was identified in the fall of 2008 at Dalhart, TX. Potato plants in the field exhibited severe psyllid yellows/ZC-like symptoms (Fig. 1A). Fifteen symptomatic plants were randomly selected and hand harvested. Foliar tissue and five tubers from each plant were collected and tested for '*Ca.* Liberibacter solanacearum' by PCR. Since potato psyllid yellows/ZC symptoms resemble those of potato purple top disease (caused by phytoplasmas), the plant and tuber samples were also tested for phytoplasma by nested PCR with universal primers P1/P7 and fU5/rU3 according to Crosslin et al. (2006). In addition, five tubers from each potato plant were checked for ZC symptoms in raw tubers and processed into fried chips according to Munyaneza

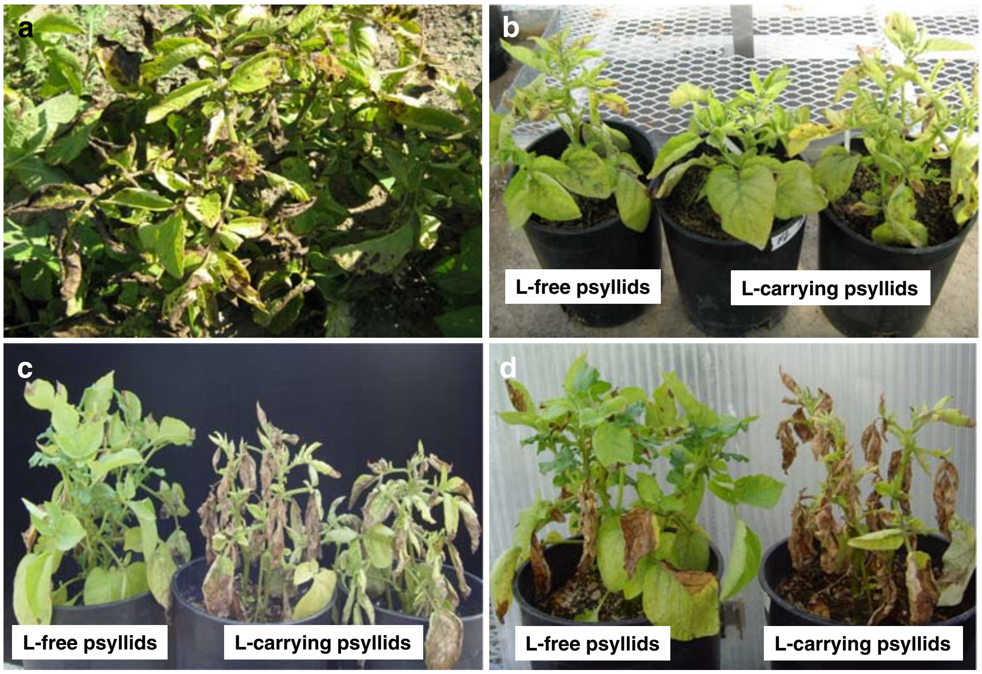

Fig. 1 Phenotypic symptom expression in potato plants affected by psyllid yellows and zebra chip (ZC) diseases. (**A**) Field collected (Dalhart, TX) potato plants exhibiting psyllid yellows/ZC-like symptoms. (**B**) Potato plants exposed to liberibacter-free and liberibacter-carrying potato psyllids and exhibiting psyllid feeding damage, 30 days after insect exposure. (**C**) and (**D**) Potato plants infected with liberibacter-free and liberibacter-carrying potato psyllids, 2–3 weeks after insect removal

et al. (2007a). PCR testing was performed at USDA-ARS in Prosser, WA whereas the chip processing was conducted at CSS Farms, Inc., Dalhart, TX.

Liberibacter Testing

Liberibacter testing by PCR was performed according to Crosslin and Munyaneza (2009). Briefly, PCR tests for all plants exposed to psyllids as well as control plants were conducted using primers OA2 and OI2c specific for 16S rDNA (Liefting et al. 2009b; Crosslin and Bester 2009; Crosslin and Munyaneza 2009; Munyaneza et al. 2009a,b,c). Nucleic acids were extracted from potatoes as described by Presting et al. (1995) or with the Wizard Genomic DNA kit (Promega, Madison, WI) as described by the manufacturer. Nucleic acids were extracted from insects using the CTAB extraction method of Zhang et al. (1998) but without grinding in liquid nitrogen. Insects (in groups of five) were ground in 600 µl of fresh buffer using a micropestle, and processed as described by Zhang et al. (1998) and nucleic acids were resuspended in 100 µl of strile water. Fifty microliter PCR reactions were conducted with Green GoTaq reaction buffer (Promega), 20 pmol of each primer, 2 µl of DNA extracts, and 1 U of GoTaq Polymerase (Promega). Reactions were incubated at 94°C for 2 min followed by 40 cycles of 94°C for 15 sec, 65°C for 30 sec, and 1 min at 72°C. Final extension for 5 min at 72°C was conducted and reactions were held at 4°C. Ten microliters of the reactions were analyzed by electrophoresis in 1.5% agarose gels, stained with ethidium bromide, and observed under ultraviolet light. Presence of the predicted 1,168 bp 16 S rDNA band indicated samples that were positive for '*Ca.* Liberibacter solanacearum'.

Results

Regardless of the psyllid colony used, all the insect-exposed plants showed psyllid damage by the third week after insect exposure (Table 1). Control plants did not exhibit any psyllid damage symptoms. Initial symptoms were characterized by plant stunting, shortened internodes, yellowing and upward curling of the leaves, pinkish discoloration and growth of lateral shoots. No apparent difference in plant symptom severity was observed among the plants exposed to either liberibacter-free or liberibacter-carrying psyllids 4–5 weeks after insect exposure (Table 1, Fig. 1B). However, plant symptom severity increased afterward in plants exposed to psyllids from both liberibacter-carrying colonies, with the plants exhibiting severe leaf scorching symptoms and sometimes producing aerial tubers. Overtime, all the plants that were exposed to liberibacter-carrying psyllids started wilting, leading to sudden death of the plants, 2 weeks after insect removal

Table 1 Phenotypic symptom expression of potato plants exposed to liberibacter-free and liberibacter-carrying potato psyllids

Duration of Psyllid exposure (days)	Psyllid exposure treatments[a]				
	Plants without psyllids (controls)	Plants exposed to liberibacter-free psyllids for 30days	Plants exposed to liberibacter-free psyllids continuously	Plants exposed to liberibacter-carrying psyllids (colony 1) for 30days	Plants exposed to liberibacter-carrying psyllids (colony 2) for 30days
10	No psyllid damage symptoms	No visible psyllid damage symptoms	No visible psyllid damage symptoms	No visible psyllid damage symptoms	No visible psyllid damage symptoms
20	Normal growth and no psyllid damage symptoms	Early psyllid damage symptoms, including leaf upward curling and yellowing	Early psyllid damage symptoms, including leaf upward curling and yellowing	Early psyllid damage symptoms, including leaf upward curling and yellowing	Early psyllid damage symptoms, including leaf upward curling and yellowing
30	Normal growth and no psyllid damage symptoms	Leaf upward curling and yellowing, shortened internodes, stunted growth, side shoot growth	Leaf upward curling and yellowing, shortened internodes, stunted growth, side shoot growth	Leaf upward curling and yellowing, shortened internodes, stunted growth, side shoot growth	Leaf upward curling and yellowing, shortened internodes, stunted growth, side shoot growth
45	Normal growth and no psyllid damage symptoms	Initial recovery, development and greening of new shoots and leaves	Severe psyllid damage symptoms with an increase in leaf yellowing and curling, shortening of internodes, and stunting	Severe leaf yellowing and purpling, leaf curling and shortening of internodes, stunting, leaf scorching, and wilting, leading to sudden plant death	Severe leaf yellowing and purpling, leaf curling and shortening of internodes, stunting, leaf scorching, and wilting, leading to sudden plant death
60	Normal growth and no psyllid damage symptoms	Increased plant recovery, with leaves and shoots growing normally	Increase in severity of psyllid damage symptoms, with severe scorching and development of aerial tubers		
75	Normal growth and no psyllid damage symptoms	Normal plant growth, similar to control plants	Gradual senescence and death of plants		
90	Gradual senescence and death of plants	Gradual senescence and death of plants			

[a] There were 15 plants per treatment; all potato plants produced tubers (2–5 tubers/plant)

(Table 1; Fig. 1C and D). In contrast, recovery and regrowth were observed in the plants exposed to psyllids from liberibacter-free colony, 2 weeks after insect removal (Table 1; Fig. 1C and D); some of the plants appeared as healthy as the controls at the end of the experiment (60 days after insect removal). However, plants exposed to liberibacter-free psyllids continuously until the end of the experiment developed severe psyllid yellows/ZC-like symptoms but did not senesce until toward the end of the experiment. Despite psyllid damage, all of the potato plants exposed to psyllids produced tubers. Typical ZC symptoms were visible and severe in raw tubers and fried chips from plants exposed to psyllids from liberibacter-carrying colonies; however, no ZC symptoms were observed in tubers produced by the plants exposed to psyllids from the liberibacter-free colony or the control plants (Table 2). PCR using OA2/OI2c primers amplified a '*Ca.* Liberibacter solanacearum'-associated 1,168 bp fragment from plant tissue and potato tubers exposed to psyllids from liberibacter-carrying colonies; however, no such amplification was detected using plant and tuber tissue extracts from potatoes exposed to psyllids from the liberibacter-free colony or control plants (Table 2, Fig. 2). In addition, PCR analysis of psyllids collected from potato plants exposed to the liberibacter-free colony 30 days after insect exposure and at the end of the experiment concluded that the insects tested negative for the bacterium. The ZC-associated liberibacter PCR amplicon was detected in psyllids collected from plants exposed to psyllids from the two liberibacter-carrying colonies (Table 2, Fig. 2).

Despite severe ZC/psyllid yellows-like symptoms observed in the potato plants collected from the Dalhart field (Fig. 1A), all plant tissue and tuber samples tested negative for liberibacter by PCR. Also, no phytoplasmas were detected in the plant tissue or tuber samples. No ZC symptoms were observed in raw tubers or fried chips.

Discussion

It is well documented that the classical psyllid yellows disease of potato is caused by the feeding of potato psyllid (Eyer and Crawford 1933; Richards and Blood 1933; Wallis 1955). Several reports on the economic importance, yield loss, and disease incidence of psyllid yellows have been published (Eyer and Crawford 1933; Wallis 1955; Arslan et al. 1985; Cranshaw 1994, 2001). ZC, another potato disease with aboveground plant symptoms resembling those of psyllid yellows, was first documented in potato fields around Saltillo, Mexico, in 1994, and was first identified in the United States in 2000 in commercial potato fields in Pearsall and the Lower Rio Grande Valley in Texas (Secor and Rivera-Varas 2004). ZC is also serious in potato

Table 2 Zebra chip (ZC) symptom assessment and PCR analysis of potato plants exposed to liberibacter-free and liberibacter-carrying potato psyllids. Also, psyllids were removed from the potato plants 30 days after insect exposure or at the end of the experiment and tested for liberibacter by PCR[a,e]

	Plants without psyllids (controls)	Plants exposed to liberibacter-free psyllids for 30days	Plants exposed to liberibacter-free psyllids continuously	Plants exposed to liberibacter-carrying psyllids (colony 1) for 30days	Plants exposed to liberibacter-carrying psyllids (colony 2) for 30days
Percent of tubers with ZC symptoms[b]	0	0	0	100	100
Percent of plants testing positive for liberibacter in foliage[c]	0	0	0	100	100
Percent of plants testing positive for liberibacter in tubers	0	0	0	100	100
Percent of potato psyllids testing positive for liberibacter[d]	–	0	0	100	100

[a] There were 15 plants per treatment; all potato plants produced tubers (2–5 tubers/plant)

[b] All produced tubers were assessed for visual ZC symptoms and tested for liberibacter by PCR.

[c] One foliar tissue sample per plant was tested for liberibacter by PCR.

[d] Three sets of five potato psyllid adults per plant were tested for liberibacter by PCR.

[e] Due to the lack of variation in ZC or liberibacter infection among treatments, no statistical analysis was performed.

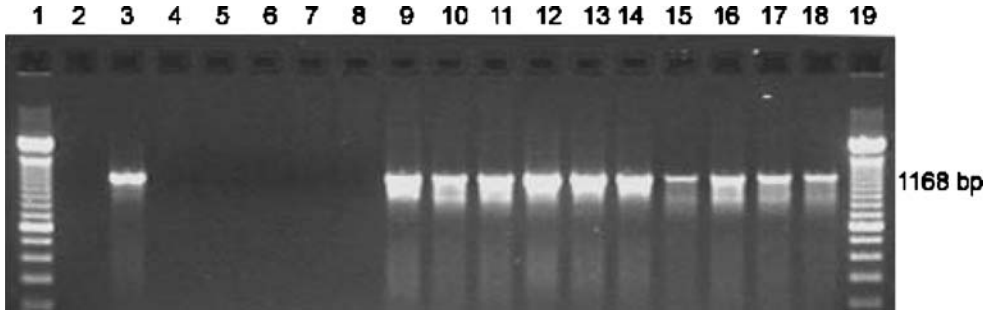

Fig. 2 PCR amplification of potato plants exposed to liberibacter-free and liberibacter-carrying potato psyllids. *Lanes 1 and 19* are 100 bp markers; healthy plant (*lane 2*); positive control (*lane 3*); plants exposed to liberibacter-free psyllids (*lanes 4–8*); plants exposed to liberibacter-carrying psyllids from colony 1 (*lanes 9–13*) and colony 2 (*lanes 14–18*)

production areas of Guatemala (Secor and Rivera-Varas 2004; Secor et al. 2009). Most recently, this emerging disease has been documented in New Zealand (Liefting et al. 2008, 2009b). Unlike pysllid yellows disease, ZC is mainly characterized by symptoms that develop in fried chips from infected potato tubers and that consist of a striped pattern of necrosis in tubers (Munyaneza et al. 2007a, b; 2008). Studies have clearly shown the association of ZC with the potato psyllid (Munyaneza et al. 2007a, b; 2008; Secor et al. 2009). ZC and psyllid yellows-infected potato plants also exhibit symptoms that resemble those of the potato purple top disease caused by the Columbia Basin potato purple top phytoplasma and transmitted by the beet leafhopper, *Circulifer tenellus* Baker (Lee et al. 2004; Crosslin et al. 2005; Munyaneza 2005; Munyaneza and Upton 2005; Munyaneza et al. 2006).

Until recently, the causal agent of ZC was not known. However, studies conducted in the United States, New Zealand, and Mexico have concluded that ZC is associated with a new species of liberibacter, '*Ca.* Liberibacter solanacearum' (Liefting et al. 2008, 2009a,b; Abad et al. 2009; Crosslin and Bester 2009; Crosslin and Munyaneza 2009; Li et al. 2009; Lin et al. 2009; Munyaneza et al. 2009a,b,c; Secor et al. 2009). Hansen et al. (2008) reported that psyllid yellows disease of potato was caused by a liberibacter species which they named '*Ca.* Liberibacter psyllaurous' (meaning psyllid yellows). This bacterium species is closely related, if not identical, to '*Ca.* Liberibacter solanacearum' (Crosslin and Bester 2009; Lin et al. 2009; Munyaneza et al. 2009a,b,c; Secor et al. 2009). However, Munyaneza et al. (2008) conducted field exposure and exclusion experiments in southern Texas using geographically different colonies of potato psyllids and the results showed that three out of nine psyllid colonies used in the study did not induce ZC symptom expression. Nevertheless, these non-ZC inducing psyllid colonies caused severe psyllid yellows-like symptoms in potato plants. These non-ZC inducing psyllid colonies were later found to be free of liberibacter (Munyaneza, unpublished data). It appears that there are distinct differences between the two potato diseases and that at least ZC is associated with liberibacter whereas psyllid yellows disease is not.

Results of the present study support the observations that ZC is consistently associated with liberibacter in contrast to psyllid yellows disease. The results showed that, although initial ZC plant symptoms resemble those resulting from continuous feeding by the potato psyllid, ZC-infected plants gradually developed severe symptoms and suddenly died after insect removal whereas plants affected by liberibacter-free psyllids fully recovered (Table 1, Fig. 1). Also, liberibacter was detected in plant tissue and tubers from all the potato plants exposed to liberibacter-carrying psyllids, but no liberibacter was present in plant tissue or tubers from plants exposed to liberibacter-free psyllids or control plants (Table 2, Fig. 2). No ZC symptoms were observed in raw tubers or fried chips from control plants or those exposed to liberibacter-free psyllids (Table 2). In contrast, typical ZC symptoms were observed in all raw tubers and fried chips from plants exposed to liberibacter-carrying psyllids (Table 2). In addition, no liberibacter or phytoplasma was detected or ZC symptoms observed in plants collected from the psyllid-infested potato field in Texas (Fig. 1A), suggesting that the observed plants symptoms were due to psyllid yellows disease and not to ZC. These results are consistent with several reports implicating liberibacter as the putative causal agent of ZC (Liefting et al. 2008; Abad et al. 2009; Crosslin and Bester 2009; Crosslin and Munyaneza 2009; Lin et al. 2009; Munyaneza et al. 2009a; Secor et al. 2009). Crosslin and Munyaneza (2009) have also shown that ZC and its putative causal agent can be maintained in potatoes by grafting and *in vitro*.

The results of the present study also support previous reports of recovery of potato plants with psyllid yellows symptoms when psyllids are removed early enough (Richards and Blood 1933; Eyer and Crawford 1933; Carter 1950; Sanford 1952; Daniels 1954; Wallis 1955; Abernathy 1991; Arslan et al. 1985), suggesting that psyllid yellows disease may not be caused by a plant pathogen. Moreover, the results of the present study support findings by Munyaneza et al. (2008) that showed that not all the psyllid populations induce ZC, but can still produce potato psyllid yellows disease symptoms and severely affect potato yield. In order to induce ZC symptoms, psyllids feeding on potatoes have to carry liberibacter. Furthermore, these results are in disagreement with the report by Hansen et al. (2008) who suggest that psyllid yellows disease of solanaceous plants, including potato, is due to liberibacter, hence the name of '*Ca*. Liberibacter psyllaurous' (i.e., psyllid yellows).

Results of the present study also revealed that ZC-affected potato plants generally died more rapidly than plants affected by psyllid yellows disease and apparently liberibacter-free. During the study, potato plants exposed to psyllids carrying liberibacter died 6–7 weeks after insect exposure and 2 weeks after insect removal (Table 1, Fig. 1C and D). However, similarly to controls, plants exposed to liberibacter-free psyllids and whose insects were removed 4 weeks after exposure fully recovered and did not senesce until the end of the experiment (Table 1). Despite exhibiting severe psyllid feeding damage symptoms, the plants exposed to liberibacter-free psyllids continuously also stayed alive until 10–11 weeks after insect exposure (Table 1). These results reflect another major difference between ZC and potato psyllid yellows disease.

In summary, it appears that there are distinctive phenotypic and etiological similarities and differences between ZC and potato psyllid yellows diseases. Results of the present study conclusively showed that aboveground plant and tuber symptoms of ZC disease are associated with liberibacter, whereas the psyllid yellows symptoms are not due to the presence of this bacterium in affected plants. In addition, tubers from liberibacter-infected potato plants exhibited symptoms that consist of a striped pattern of necrosis in fried chips, a typical characteristic of ZC infection, but tubers from psyllid yellows-affected plants did not. Furthermore, ZC-infected plants tend to die quickly, in contrast to potato plants affected by psyllid yellows disease. Although an association between liberibacter and ZC has been established, the causal agent(s) of classical psyllid yellows remain unknown and mechanisms by which the potato psyllid induces the disease symptoms are not well understood.

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